IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Kasahara, et al. Art Unit : 1633
Serial No. : 10/045,178 Examiner : Ileana Popa
Filed : January 11, 2002
Title : GENE DELIVERY SYSTEM AND METHODS OF USE

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF NORIYUKI KASAHARA UNDER 37 C.F.R. § 1.132

Dear Sir:

1. I, Noriyuki Kasahara, declare and state that I am a resident of Los Angeles, California. My residence address is 8446 Kirkwood Drive, Los Angeles, California 90046.

2. I have a Bachelor degree in Medical Science that I received from Tokyo Medical and Dental University, in 1986. I further hold an M.D. degree that I received from Tokyo Medical and Dental University in 1993, and a Ph.D. degree that I received from the University of California, San Francisco (UCSF) in 1994. I received Board certification in the field of Clinical Pathology from the American Board of Pathology in 1996. I am currently an Associate Professor in the Department of Medicine at the University of California, Los Angeles (UCLA) David Geffen School of Medicine, 675 Charles E. Young Drive South, MRL-1551, Los Angeles, CA. I am an expert in the fields of clinical pathology, molecular biology, virology, virus-based gene delivery vector technology development, and gene therapy.

3. I am an inventor of the claims of the above-identified patent application.

4. I understand that the Examiner has rejected the pending claims in this application on the grounds that the specification allegedly fails to provide an enabling disclosure. In my opinion, the specification sufficiently enables the pending claims for the following reasons.

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5. The specification of the present application describes replication-competent retrovirus (RCR) vectors. For example, Example 1 describes a construction method of RCR vectors. The ACE-GFP RCR vector of parts 6 and 7 of the present declaration, the ACE-CD RCR vector of part 8 of the present declaration, and the ACE-hIL2 RCR vector of part 9 of the present declaration, were prepared according to the methods discussed in Example 1. ACE-GFP contains the marker gene encoding green fluorescent protein (GFP), ACE-CD contains the suicide gene encoding cytosine deaminase (CD), and ACE-hIL2 expresses human interleukin 2 (IL-2).

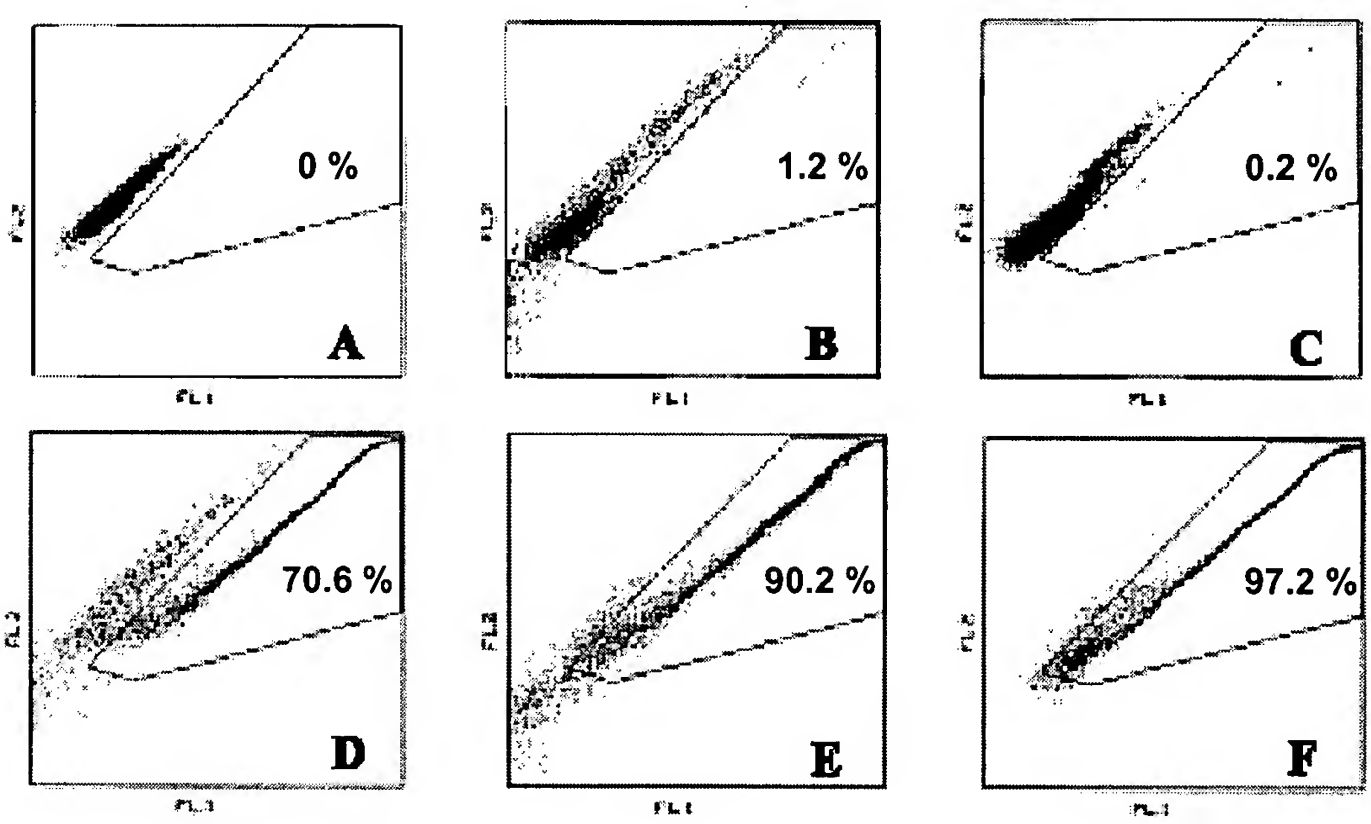
6. The RCR vectors of the present application achieve efficient transgene delivery to solid tumors in vivo. As described in Example 9 of the present application, the g1ZD-GFP vector was inoculated into a tumor in an animal model at a very low dose (multiplicity of infection (MOI) calculated to 0.1% or less). Cells of the tumor were isolated at various time and GFP expression was examined by FACS analysis. The results indicate that the vector resulted in highly efficient and stable transduction, especially in view of the low MOI. Thus, starting with an initial low percentage of transduced cells, the virus spread to the entire tumor in the six weeks tested.

ACE-GFP and ACE-CD were also tested in the same manner as described in Example 9. U-87 human glioma cells (5×10^5 cells) were first implanted subcutaneously into athymic nu/nu mice. Tumors were allowed to grow up to 0.5 cm in diameter, then PBS vehicle control, the amphotropic RCR vector ACE-GFP (1.2×10^5 TU/100 μl), or a conventional replication-defective retrovirus vector expressing GFP (1.0×10^5 TU/100 μl) was injected into the tumor. After sacrifice at serial time intervals 2, 4, and 6 weeks after vector inoculation, quantitation of GFP expression in the transduced tumors was performed by FACS analysis immediately after dissection and collagenase digestion to obtain a single-cell suspension of the tumor sample.

FACS analysis of replication-defective vs. RCR vector-transduced subcutaneous gliomas is provided in Figure 1. The percentage of GFP positive cells in the freshly dissected tumors that had been infected by the replication-defective vector was low, only 1.2% at 2 weeks, and 0.2% at

6 weeks post-vector injection (Figure 1, B-C), consistent with the results observed in clinical trials. In contrast, the percentage of GFP positive cells infected by the replication-competent retrovirus (ACE-GFP) was 70.6%, 90.2%, and 97.2% at 2, 4, and 6 weeks post-injection, respectively (Figure 1, D-F). These results demonstrate that, in contrast to conventional replication-defective vectors, the RCR vector was capable of essentially complete transduction of the entire U-87 tumor mass within 6 weeks.

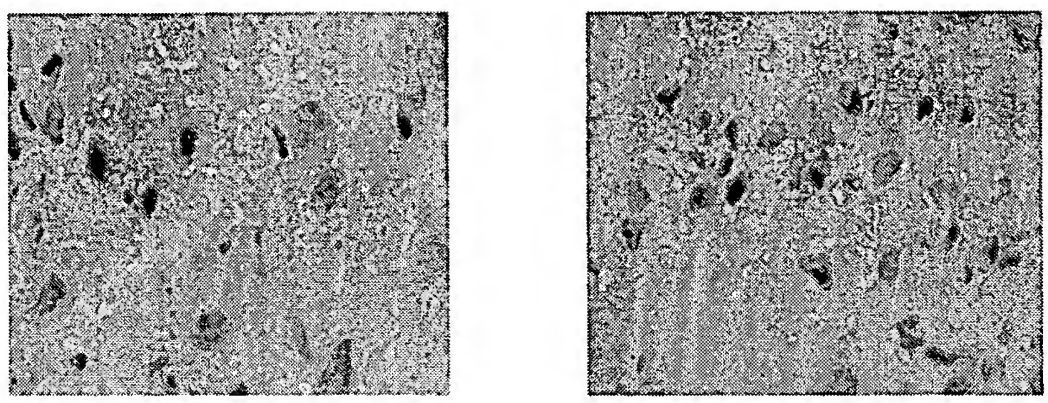
Figure 1



7. We have further tested the RCR vector for infectivity of non-target tissue. We have found that, even when directly injected into normal rat brain, the vector failed to transduce quiescent normal cells. The MLV-based RCR vector (ACE-GFP) (1.2×10^4 TU/10 μl) was injected directly into the right frontal lobe of normal mice, and GFP expression assessed by immunohistochemistry. No GFP signal was detected in normal brain tissue injected with ACE-GFP (Figure 2) (A) Section of normal brain injected with PBS, or (B) ACE-GFP (1.2×10^4 TU) into the right frontal lobe of euthymic mice. Both sections were processed for GFP immunohistochemistry.

Figure 2

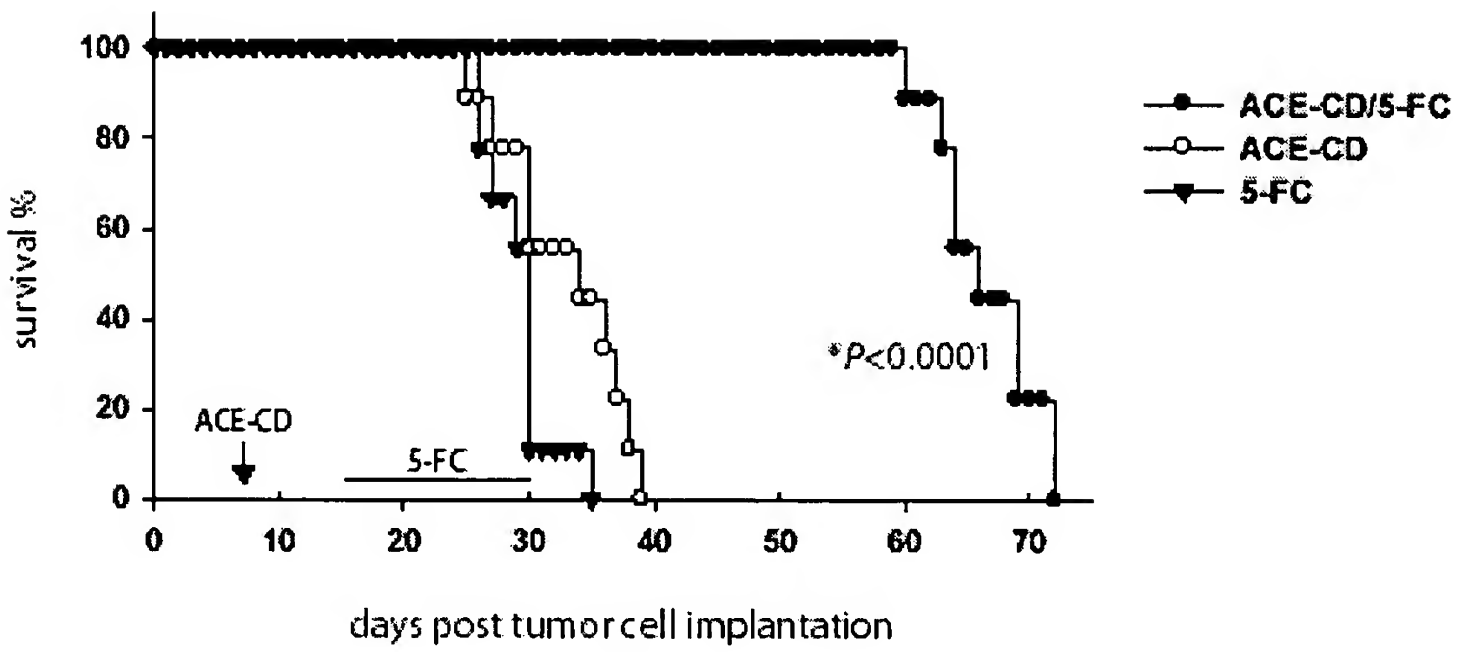
A **B**



8. We have further found that methods employing the novel replication competent retrovirus significantly prolong survival of athymic mice implanted with U-87 intracerebral gliomas. An RCR vector expressing the yeast cytosine deaminase suicide gene was tested in the intracranial U-87 glioma model. Yeast cytosine deaminase converts the non-toxic pro-drug 5-fluorocytosine (5-FC) to the toxic metabolite 5-fluorouracil (5-FU), which acts as an intracellular chemotherapeutic agent to kill transduced tumor cells.

One week after tumor implantation, approximately 1.0×10^4 TU of the vector in a total volume of 10 μl was stereotactically injected into intracranial U-87 tumors in 2 groups of mice (n = 9 each). An additional group (n = 9) received only PBS vehicle control. Eight days after vector transduction, the 5-FC prodrug, 500 mg/kg/day, was given for 15 consecutive days by daily intraperitoneal injection to one of the ACE-CD injected groups and to the PBS vehicle injected group. The remaining ACE-CD injected group received only daily intraperitoneal injections of PBS for 15 consecutive days. The mice treated with ACE-CD plus a single cycle of 5-FC prodrug showed a doubling of median survival over a follow-up period of more than 70 days (See Figure 3), compared to mice treated with either ACE-CD/PBS ($p<0.0001$) or PBS/5-FC prodrug ($p<0.0001$).

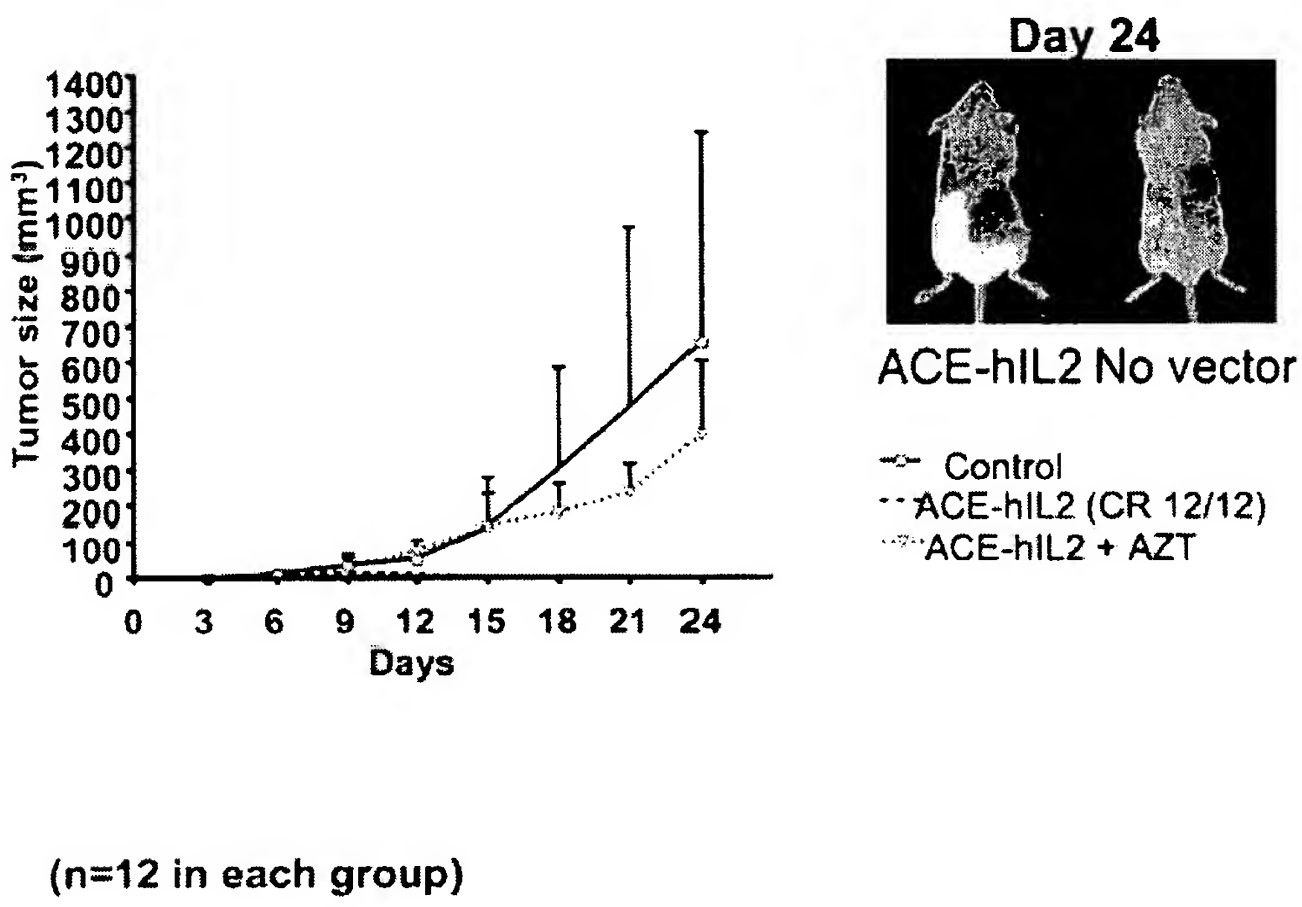
Figure 3



9. We also tested the therapeutic effect of an RCR vector in the treatment of multiple liver metastases in an animal model using immunocompetent Balb/c mice. In this model, subcutaneous tumors were established by injecting CT26 (a mouse colon cancer cell line) cells into Balb/c mice. ACE-hIL2, an RCR vector containing the human interleukin 2 (IL-2) gene, was expressed in 2% of the CT26 cells. As shown in Figure 4, control animals that did not receive any vector (solid line) developed tumors rapidly. In contrast, the animals that received ACE-hIL2 (dashed line, which almost overlaps with the base line) developed tiny tumors in the beginning (around 6-9 days), but all the tumors went into regression. As indicated by the note "CR 12/12" in the figure legend on the right-hand side for the ACE-hIL2 group, 12 out of 12 animals in this group had a complete response (complete tumor regression). A picture of two animals on Day 24, one from the ACE-hIL2 group and the other from the control group, clearly shows the difference in tumor size (note that the fur overlying the tumor sites of both animals has been shaved for better visualization).

This therapeutic effect depended on replication of the ACE-hIL2 RCR vector. When the ACE-hIL2 group received azidothymidine (AZT), which inhibits retroviral replication, the anti-tumor effect of ACE-hIL2 was greatly reduced (dotted line in Figure 4).

Figure 4



(n=12 in each group)

These results thus demonstrate that RCR vectors can be used to deliver cytokines to effectively inhibit tumor formation.

10. The specification of the above-referenced patent application provides ample guidance to the person of ordinary skill in the art to successfully practice the claimed invention. For example, one skilled in the art at the time the application was filed would know, given the information provided in the specification, that treatment of a subject with an RCR vector of the invention would likely result in a therapeutic effect.

11. All of the experiments described in the specification, in conjunction with above experiments which were performed according to methods and the examples described in the specification, demonstrate that the claimed invention is useful for efficiently transferring a therapeutic polypeptide to a large number of neoplastic cells in vivo. The experiments further indicate that, post-infection, the RCR vector produces therapeutic levels of a pro-drug or cytokine in a subject. Using the methods and compositions described in the specification, one of ordinary skill in the art would have a reasonable expectation that the claimed invention would be applicable to providing a expression of a pro-drug or cytokine in neoplastic cells for the purpose of treating a cell-proliferative disorder in a subject in need of such treatment.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date: 7/28/06

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